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(54) Title: DETERMINATION OF THE RATIO OF ABSORPTION COEFFICIENTS AT DIFFERENT WAVELENGTHS IN A SCATTERING MEDIUM (57) Abstract <p>A method of determining the ratio of the absolute absorption coefficients $\mu_a(\lambda_2)$ and $\mu_a(\lambda_1)$ of a light scattering medium at two different wavelengths comprising: a) passing light (as herein defined) through the medium; b) measuring a characteristic X of the light at two wavelengths λ_1 and λ_2 which is affected by the absorption coefficient during its passage through the medium; c) effecting changes $\Delta\mu_a(\lambda_1)$ and $\Delta\mu_a(\lambda_2)$ in the absorption coefficients at the two wavelengths; d) repeating step (b); e) determining the ratio of the absolute absorption coefficients $\mu_a(\lambda_1)$ and $\mu_a(\lambda_2)$ at said two wavelengths from the said changes therein $\Delta\mu_a(\lambda_1)$ and $\Delta\mu_a(\lambda_2)$ or from quantities representative thereof, from the characteristic X, and from the ratio of transport scattering coefficients of the medium $\mu_s'(\lambda_1)$ and $\mu_s'(\lambda_2)$ at the two wavelengths.</p> <div data-bbox="743 1549 1024 1808" style="border: 1px solid black; padding: 5px; margin: 20px auto; width: fit-content;"><div>FHP 99-42EP</div><div>02.6.26</div><div>SEARCH REPORT</div></div>		

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DETERMINATION OF THE RATIO OF ABSORPTION COEFFICIENTS AT DIFFERENT WAVELENGTHS IN A SCATTERING MEDIUM

This invention relates to the determination of the ratio of absorption coefficients at different wavelengths in a scattering medium, and to the determination of the relative concentrations of chromophores in the medium.

There are a number of industrial processes in which measurement of the ratio of chromophore concentrations in a scattering medium would (if available) be an important indicator for process quality control. Examples are the measurement of water, protein and fat content in liquids and solids in bioreactors and food processing, and the measurement of relative dye concentrations in dyeing processes, which relative concentrations are critical to the consistency of the final colour achieved in the dyed product.

Other industrial applications would be the measurement of the ratio of chromophore concentrations in tissue samples, for example relative oxy- and deoxy haemoglobin concentrations. Measurement of such quantities in vivo would also be useful in support of clinical practice, as would measurement of the oxygen saturation of blood in tissue.

It is an object of at least the preferred embodiment of the invention to provide a method of determining relative concentrations of chromophores in a scattering medium, and also to provide a method of determining relative absorption coefficients of the medium itself, since this may be sufficient for some applications.

In a first aspect the invention provides a method of determining the ratio of the absorption coefficients $\mu_a(\lambda_1)$ and $\mu_a(\lambda_2)$ of a light scattering medium at two different wavelengths comprising

- a) passing light (as herein defined) through the medium
- b) measuring a characteristic X of the light at two wavelengths λ_1 and λ_2 which is affected by the absorption coefficient during its passage through the medium
- c) effecting changes $\Delta\mu_a(\lambda_1)$ and $\Delta\mu_a(\lambda_2)$ in the absorption coefficient at the two wavelengths;
- d) repeating step (b)
- e) determining the ratio of the absorption coefficients $\mu_a(\lambda_1)$ and $\mu_a(\lambda_2)$ at said two wavelengths from said changes therein or from quantities representative thereof and from the

characteristic X and from the transport scattering coefficients of the medium $\mu_s'(\lambda_1)$ and $\mu_s'(\lambda_2)$ at the two wavelengths.

The term "light" as used herein includes the near infrared spectrum.

The said changes in the absorption coefficient may be effected by a common cause, for example by varying the concentration of a chromophore in the scattering medium, the quantities representative of the changes in the absorption coefficient being the absorption coefficients $\Delta\mu_a(\lambda_1)$, $\Delta\mu_a(\lambda_2)$. In tissue, these changes can be deliberately generated by injecting an exogenous dye (e.g. Indocyanine green) intravenously or modifying haemoglobin oxygenation or volume.

The method may comprise first adding to the medium the chromophore where concentration is varied.

The ratio of the absorption coefficients $\mu_a(\lambda_1)$ and $\mu_a(\lambda_2)$ may be determined from the ratio of the chromophore absorption coefficients $\Delta\mu_a(\lambda_1)$, $\Delta\mu_a(\lambda_2)$ and from the ratio of the transport scattering coefficients of the scattering medium $\mu_s'(\lambda_1)$ and $\mu_s'(\lambda_2)$.

In one embodiment the characteristic measured in step (b) may be the attenuation A of the light during its passage through the medium.

In another embodiment the light may be intensity modulated or pulsed light, the measured characteristic X being the phase or depth of the modulation or the time of flight (transmission time) of the light through the medium respectively.

The invention also provides a method of determining the variation with wavelength of the absorption coefficient of a scattering medium (the spectral characteristic) comprising performing the method as set forth above at a number of wavelengths across a waveband of interest, and in step (e) thereof determining the ratio of the absorption coefficients relative to that at a reference wavelength λ_r , the variation of said ratios with wavelength being the said spectral characteristic.

In a further aspect the invention provides a method of determining the ratio of chromophore concentrations of known spectral characteristics (as herein defined) in a light scattering medium comprising performing the method of steps (a) to (d) as set forth above at not less than n different wavelengths where n is the number of chromophores, one of said wavelengths being the reference wavelength λ_r , performing step (e) to determine the ratio $\mu_a(\lambda_1)/\mu_a(\lambda_r)$ of the medium for each of the other wavelengths, and determining the relative concentrations of the chromophores in the medium from said ratios.

By spectral characteristic we mean the variation of μ_a with λ .

Where the absolute value of the absorption coefficient of one of the chromophores is known (eg water) or can be found by other means, the method will yield the absolute spectral characteristics and hence absolute concentrations of the other chromophores.

The invention also includes apparatus for use in the method as set forth above.

The invention will now be described merely by way of example with reference to the accompanying figures, wherein

Figure 1 shows the fractional error in the ratio $\mu_a(\lambda_1)/\mu_a(\lambda_2)$ from the approximation in equations 3 and 4, discussed below. Specifically, it shows fractional error in ratio $\mu_a(\lambda_2)/\mu_a(\lambda_1)$ from approximations of equations 3 and 4 assuming $\mu_a(\lambda_1) = 0.02 \text{ mm}^{-1}$, $\mu_s'(\lambda_2) = \mu_s'(\lambda_1) = 1 \text{ mm}^{-1}$ and a source detector $r = 50 \text{ mm}$.

Figure 2a shows the optical properties of a liquid tissue-simulating phantom used in an experiment described hereafter. The absorption coefficient is that of water ($\mu_{a,w}$) and the added dye ($\mu_{a,d}$). The transport scattering coefficient (μ_s') is based upon calculations from Mie theory for the concentration $c_s = 2\%$ and the diameter ($1 \mu\text{m}$) of the spheres.

Figure 2b shows the spectrum of the absorption coefficient of the phantom normalised at $\lambda = 800 \text{ nm}$. Specifically, it shows spectrum of the relative absorption coefficient ($\mu_a(\lambda)/\mu_a(800\text{nm})$) of the phantom normalised at $\lambda = 800\text{nm}$. The dotted line shows the true values derived from the water and dye absorption of the phantom ($\mu_{a,w} + \mu_{a,d}$ in figure 2a). The measured attenuation changes that were induced by a small increase in $\mu_{a,d}$ were used to estimate $\mu_a(\lambda)/\mu_a(800\text{nm})$ by assuming a constant μ_s' (dashed line, calculated from Eq. 6 below) and by including the wavelength dependence of μ_s' (solid line, Eq. 7).

Figure 3 shows values of the transport scattering coefficient μ_s' measured on the heads of seven volunteers. The values are an estimate from measured TPSFs (data from Matcher et al 1996).

Figure 4 shows values of μ_s' measured on the calf muscle of eleven volunteers; μ_s' measured on the head of 7 volunteers. The values are an estimate from measured TPSFs (data from Matcher et al 1996).

Figure 5 shows apparatus according to the invention.

Changes in the chromophore concentration ie. changes in the absorption coefficient ($\Delta\mu_a$) in a light scattering medium alter the intensity of the diffusely transmitted light collected at the surface of the medium. Here we describe how the spectral characteristic of the tissue absorption coefficient can be calculated from the known spectral characteristic of an injected dye absorption coefficient and the change in the measured spectrum of the intensity (attenuation), phase shift or mean time.

Once the spectral characteristic of the tissue absorption coefficient has been determined, the relative concentrations of its chromophores, notably the concentrations of oxy- and deoxy - haemoglobin (HbO_2 and Hb), can be calculated. An application is the monitoring of the oxygen saturation ($\text{HbO}_2/(\text{HbO}_2 + \text{Hb})$) of blood in tissue.

Diffusion Theory Model

For a semi-infinite halfspace the reflectance at a distance r from the light source can be written as

$$R(r) = z_0 \cdot \left(\frac{1}{\rho} + \mu_{eff} \right) \cdot \frac{\exp(-\mu_{eff} \cdot \rho)}{2\pi \cdot \rho^2} \quad (1)$$

In Eq. 1 $\rho = (r^2 + z_0^2)^{1/2}$ and $z_0 = 1/\mu_s'$, where μ_s' is the transport scattering coefficient. The velocity of light in the medium $c = c_0/n$ (where the speed of light in vacuum is c_0).

$\mu_{eff} = (3 \cdot \mu_a (\mu_a + \mu_s'))^{1/2}$ is the effective attenuation coefficient and $D = (3 \cdot (\mu_a + \mu_s'))^{-1}$ is the diffusion coefficient. The derivative of the attenuation A (defined as the logarithm of the

ratio of incident and detected intensity) with respect to change in μ_a is:

$$\frac{\partial A}{\partial \mu_a} = \frac{3}{2 \cdot \ln 10} \cdot \frac{\rho}{1/\rho + \mu_{eff}} \cdot (2\mu_a + \mu_s') \quad (2)$$

Here it is suggested that measurements of $\partial A / \partial \mu_a$ can be used to estimate the product of absorption and scattering coefficient. For a known wavelength dependence of μ_s' , the spectral shape of μ_a can then be calculated from measurements of $\partial A / \mu_a$ at different wavelengths.

Assuming that scattering dominates absorption ($\mu_s' \gg \mu_a$) Eq. 2 can be approximated to:

$$\mu_a = \left[\frac{3\rho \cdot \mu_s'}{2 \cdot \ln 10} \cdot \left(\frac{\partial A}{\partial \mu_a} \right)^{-1} - \frac{1}{\rho} \right]^2 \cdot \frac{1}{3\mu_s'} \quad (3)$$

Furthermore, for large source-detector distances it is:

$$\mu_a = \left[\frac{\rho}{2 \cdot \ln 10} \cdot \left(\frac{\partial A}{\partial \mu_a} \right)^{-1} \right]^2 \cdot 3\mu_s' \quad (4)$$

Eq. 4 states that the ratio of the absorption coefficients at different wavelengths (λ_1, λ_2) can be estimated from measurements of attenuation changes (ΔA) at these wavelengths, the ratio of the changes in absorption coefficient and the wavelength dependence of μ_s' .

$$\frac{\mu_a(\lambda_1)}{\mu_a(\lambda_2)} = \left[\frac{\Delta A(\lambda_2)}{\Delta A(\lambda_1)} \cdot \frac{\Delta \mu_a(\lambda_1)}{\Delta \mu_a(\lambda_2)} \right]^2 \cdot \frac{\mu_s'(\lambda_1)}{\mu_s'(\lambda_2)} \quad (5)$$

To estimate the validity of the approximations leading from Eq. 2 to Eqs 3 and 4, the errors in the calculated ratio $\mu_a(\lambda_2)/\mu_a(\lambda_1)$ are plotted in Fig. 1 as a function of the true $\mu_a(\lambda_2)/\mu_a(\lambda_1)$ assuming $\mu_a(\lambda_1) = 0.002 \text{ mm}^{-1}$, $\mu_s'(\lambda_2) = \mu_s'(\lambda_1) = 1 \text{ mm}^{-1}$ and a source-detector distance $r = 50 \text{ mm}$. In the figure the fractional errors of Eqs 3 and 4 are shown in solid and dotted line, respectively. The fractional errors increase with increasing differences of $\mu_a(\lambda_2)$ and $\mu_a(\lambda_1)$. Variations in the magnitude of μ_a of a factor of 2 result in errors of approximately 10%.

Determination of the relative absorption spectrum of a light-scattering phantom

The method suggested above was tested in a liquid, light scattering tissue simulating phantom of known optical properties. Silica spheres (Monospher M1000, Merck, Germany) of 1 μm diameter were suspended in water and served as scattering centres in the phantom. The scattering properties could be derived from Mie theory calculations as the diameter of the spheres and the refractive index of both the spheres and the surrounding medium are known. The calculated scattering cross section of the spheres was compared with measurements from a collimated beam setup. Experimental and theoretical scattering cross sections were in agreement when the wavelength dependence of the refractive index of fused silica ($n_s = 1.447 + 3890\lambda^{-2} - 37\lambda^{-4}$ with λ in nm) and water ($n_w = 1.32 + 6878\lambda^{-2} - 1.132 \cdot 10^{-9} \lambda^4$) were employed. The transport scattering coefficient μ_s' was calculated for wavelengths between 600 and 1050 nm for these refractive indices and the concentration of the spheres in the phantom ($c_s = 2\%$ vol./vol.). The calculated μ_s' decreases approximately linearly with wavelength and a first order regression through the calculated values gave $\mu_s' = 0.92 - 0.00047 \lambda [\text{mm}^{-1}]$ (λ in nm). The absorption coefficient of the phantom consisted of two components: the water absorption ($\mu_{a,w}$) and the absorption of a dye (S109564, ICI, GB) ($\mu_{a,d}$) added at a concentration of $c_d = 0.0031\%$ vol./vol.. The spectra of both μ_s' and μ_a are shown in Fig. 2a.

A CCD spectrometer in connection with a halogen light source was employed to measure the reflected light intensity of the phantom (volume 300 ml). The ends of the optical fibres that transported the light from the lamp to the phantom and from the phantom to the detector were submerged approximately 2 mm in the phantom at a source-detector distance of $r = 38$ mm. Two spectra (I_0 and I) of the reflected light intensity were recorded before and after the concentration of the dye was changed by $\Delta c_d = 2.5 \times 10^{-4}\%$. This corresponds to a change in μ_a of between 4% and 8% depending of wavelength. The spectrum of the attenuation change $\Delta A(\lambda) = \log[I_0(\lambda)/I(\lambda)]$ was calculated. Ignoring the wavelength dependence of scattering gives a first approximation of the spectral characteristic $\mu_a(\lambda)$ according to Eq. 4/5, normalised a) for the relative magnitude of the absorption change (ie. divided by the absorption spectrum of the dye $\mu_{a,d}$) and b) for the value at $\lambda = 800$ nm:

$$\frac{\mu_a(\lambda)}{\mu_a(800\text{nm})} = \left[\frac{\Delta A(800\text{nm})}{\Delta A(\lambda)} \cdot \frac{\mu_{a,d}(\lambda)}{\mu_{a,d}(800\text{nm})} \right]^2 \quad (6)$$

This first approximation of $\mu_a(\lambda)$ (dashed line in Fig. 2b) shows considerable discrepancies with the true $\mu_a(\lambda)/\mu_a(800\text{nm})$ spectrum (dotted line). Correcting the experimental data for the wavelength dependent scattering coefficient of the phantom ($\mu_s'(\lambda) = 0.92 \text{ mm}^{-1} - 0.00047\lambda[\text{mm}^{-1}]$).

$$\frac{\mu_a(\lambda)}{\mu_a(800\text{nm})} = \left[\frac{\Delta A(800\text{nm})}{\Delta A(\lambda)} \cdot \frac{\mu_{a,d}(\lambda)}{\mu_{a,d}(800\text{nm})} \right]^2 \cdot \frac{\mu_s'(\lambda)}{\mu_s'(800\text{nm})} \quad (7)$$

gives the spectrum shown plotted with a solid line in Fig. 2b. The experimental data are in good agreement with the expected μ_a spectrum for $\lambda < 950 \text{ nm}$. For longer wavelengths the experimental data are noisy and underestimate μ_a . The poor signal to noise ratio for these wavelengths is due to the low sensitivity of the CCD detector and the higher absorption coefficient of the phantom. The likely explanation for the underreading at these wavelengths is, as demonstrated in Fig.1, that the approximations used for the derivation of Eq. 3-5 break down for larger ratios $\mu_a(\lambda)/\mu_a(800\text{nm})$.

Calculation of relative chromophore concentrations from the relative absorption spectrum

In the experiment, the determination of ratio of chromophore concentrations and the oxygenisaturation of blood depends on the following points:

- a) Attenuation spectra can be corrected for the wavelength dependence of μ_s' : Figure 3 and 4 show μ_s' of the head and the calf muscle from volunteers obtained from measured temporal point spread functions (TPSFs). The absolute μ_s' varies by up to 30% for different volunteers, however it has a similar wavelength characteristic. Therefore, a correction of the attenuation spectra for μ_s' variations is feasible.
- b) A pure change in absorption coefficient occurs and the wavelength dependence of the absorption coefficient change is known. Absorption changes can be induced by an exogenous dye or by changes of haemoglobin concentration. Change in Hb or HbO₂ can be generated by small changes in the inspired gas. Alternatively, the absorption

coefficient can be reduced by dilution (for example by injecting saline).

- c) The spectral characteristic of all tissue chromophores is known:
For tissue and wavelengths in the near infrared the dominant chromophores are deoxy- and oxyhemoglobin and water, the absorption spectra of which are known.
In general the absorption coefficient at a wavelength λ can be written as

where ϵ_n (unit: $\text{mm}^{-1} \cdot \text{mM}^{-1}$) is the extinction coefficient of the n^{th} chromophore and c_n is its concentration (unit: mM). Once the ratio $\mu_a(\lambda)/\mu_a(\lambda_r)$ at least at, n wavelengths has been determined (λ_r is a reference wavelength), an iterative technique can be used to calculate the relative chromophore concentrations c_n/c_R (where c_R signifies the concentration of a reference chromophore).

The number of wavelengths has to be equal to or higher than the number of chromophores in the medium. The absorption spectrum of the dye used to induce attenuation changes has to be known. For the determination of the relative oxy- /deoxy haemoglobin concentrations in tissue, the dye could for example be Indocyanine green.

In the discussion above measured changes in the light intensity were used. Equally changes in the time of flight, the phase shift or the modulation depth of an intensity modulated light wave can be exploited.

Thus changes in the mean (transit) time ($\langle t \rangle$) of the photons or the phase (Φ) of an intensity light wave (modulated at the frequency ν_M) can be used to compute the spectral characteristic of μ_a .

The mean transit time is given by

$$\langle t \rangle = \frac{\rho^2}{2c \cdot (D + \rho \cdot \sqrt{\mu_a \cdot D})} \quad (8)$$

and the phase is approximately a linear function of $\langle t \rangle$:

$$\Phi = -2\pi \cdot v_M \cdot \langle t \rangle \quad (9)$$

The derivation of $\langle t \rangle$ with respect to μ_a can be approximated as

$$\frac{\partial \langle t \rangle}{\partial \mu_a} = \frac{-\rho}{4 \cdot c} \sqrt{3} \cdot \sqrt{\frac{\mu_s'}{\mu_a}} \cdot \frac{1}{\mu_a} \quad (10)$$

Therefore,

$$\mu_a = \left[\left(\frac{\rho}{4 \cdot c} \right)^2 \cdot \mu_s' \cdot \left(\frac{\partial \langle t \rangle}{\partial \mu_a} \right)^{-2} \right]^{1/3} \quad (11)$$

The equivalent to Eq.5 with respect to measured changes in mean transit time is therefore

$$\frac{\mu_a(\lambda_1)}{\mu_a(\lambda_2)} = \left(\left[\frac{\Delta \langle t \rangle(\lambda_2)}{\Delta \langle t \rangle(\lambda_1)} \cdot \frac{\Delta \mu_a(\lambda_1)}{\Delta \mu_a(\lambda_2)} \right]^2 \cdot \frac{\mu_s'(\lambda_1)}{\mu_s'(\lambda_2)} \right)^{1/3} \quad (12)$$

The measurements of phase changes $\Delta\Phi$, Eq. 12 can be combined with Eq. 9 to give

$$\frac{\mu_a(\lambda_1)}{\mu_a(\lambda_2)} = \left(\left[\frac{\Delta \Phi(\lambda_2)}{\Delta \Phi(\lambda_1)} \cdot \frac{\Delta \mu_a(\lambda_1)}{\Delta \mu_a(\lambda_2)} \right]^2 \cdot \frac{\mu_s'(\lambda_1)}{\mu_s'(\lambda_2)} \right)^{1/3} \quad (13)$$

The modulation depth can be written as

$$M = \frac{\sqrt{1 + \psi_0^2 + 2\psi_i}}{1 + \psi_\infty} \cdot \exp(\psi_\infty - \psi_i)$$

where $\psi_0 = \mu_{eff} \cdot \rho [1 + \chi^2]^{1/4}$, $\psi_i = -\psi_0 \cdot \sin(\theta/2)$,

$\psi_i = \psi_0 \cdot \cos(\theta/2)$, $\theta = \tan^{-1}(\chi)$, $\psi_\infty = \mu_{eff} \cdot \rho$ and

$\chi = (2\pi v_M)/(\mu_a c)$.

Changes in μ_a induce changes ΔM . It can be shown that equivalently to Eq.7, 12 and 13 the

ratio of absorption coefficients at two different wavelengths can be derived from the measured fractional change $\delta M = \Delta M/M$ at these wavelengths according to

$$\frac{\mu_a(\lambda_1)}{\mu_a(\lambda_2)} = \left(\left[\frac{\delta M(\lambda_2)}{\delta M(\lambda_1)} \cdot \frac{\Delta \mu_a(\lambda_1)}{\Delta \mu_a(\lambda_2)} \right]^2 \cdot \frac{\mu'_s(\lambda_1)}{\mu'_s(\lambda_2)} \right)^{1/5}$$

Figure 5 shows diagrammatically apparatus according to one embodiment of the invention in which changes in attenuation of light passing through the medium are measured. A medium 10 under examination receives light from a white light source 12 via an optical fibre 14.

Diffused light is received by an optical fibre, and is taken to a wavelength dispersive device 18 and thence to a detector 20. The output of the detector 20 is fed to a PC or other data processor 22.

The wavelength dispersive device, for example a spectrometer comprising an optical slit and a diffraction grating divides up the white light issuing from the medium into its constituent wavelengths, those of interest then being selected by output slits or by being detected by respective elements if the detector 20 is of the staring-array type. The detector 20 may be a charge-coupled device or an array of photodiodes.

Provided the intensity of the light source 12 is kept constant, it is unnecessary to provide the detector 20 with a measure of the input light intensity. All necessary data is contained in changes in intensity arising from imposed changes in λ and μ_a .

As an alternative to the spectrometer, a wavelength selective device may be employed such as a rotatably mounted filter wheel with interference filters with two or more coloured filter elements for passing the wavelengths of interest.

The wavelengths of interest are typically in the range 650nm to 1000nm for tests on tissue. When the distance between light source and detector fibers is small (<5mm) wavelengths of up to about 2000nm (2 μ m) are appropriate. Preferably the wavelength dispersive or selective device should have a bandwidth of less than 10 nm.

In an alternative embodiment the white light source 12 is replaced by switchable or

otherwise selectable monochromatic light sources (eg laser diodes or LEDs) of different wavelengths, the wavelength dispersive or selective device 18 then not being required. Laser diodes are preferred to LEDS because the wavelength of the emitted light is more monochromatic.

When the invention is to be implemented by measuring the time of flight $\langle t \rangle$, a pulsed laser diode is used as the source 12 to provide pulsed light of a specific wavelength to the medium 10.

The wavelength dispersive device 18 is omitted and the detector 20 is replaced by means to determine the time of arrival of the pulse. A signal is provided via line 24 from the drive circuitry of the laser diode to indicate the time of initiation of the pulse to the detector so that $\langle t \rangle$ may be established.

For implementing the invention by measuring the phase shift $\Delta\phi$ or depth of modulation of light, light source 12 is an intensity modulated monochromatic source with a choice of frequencies, either switchable laser diodes or LEDs, or a white light source with several alternative filters. The detector 20 is phase-sensitive, and receives a reference signal from the input to the sample via a line 26. Phase detection may conveniently be by a lock-in technique in which the output from the medium is compared with the input from line 26 by accurately multiplying the detector signal by both the reference signal and the reference signal shifted in phase by 90° .

Each feature disclosed in this specification (which term includes the claims) and/or shown in the drawings may be incorporated in the invention independently of other disclosed and/or illustrated features.

CLAIMS

1. A method of determining the ratio of the absolute absorption coefficients $\mu_a(\lambda_2)$ and $\mu_a(\lambda_1)$ of a light scattering medium at two different wavelengths comprising
 - a) passing light (as herein defined) through the medium
 - b) measuring a characteristic X of the light at two wavelengths λ_1 and λ_2 which is affected by the absorption coefficient during its passage through the medium
 - c) effecting changes $\Delta\mu_a(\lambda_1)$ and $\Delta\mu_a(\lambda_2)$ in the absorption coefficients at the two wavelengths;
 - d) repeating step (b)
 - e) determining the ratio of the absolute absorption coefficients $\mu_a(\lambda_1)$ and $\mu_a(\lambda_2)$ at said two wavelengths from the said changes therein $\Delta\mu_a(\lambda_1)$ and $\Delta\mu_a(\lambda_2)$ or from quantities representative thereof, from the characteristic X, and from the ratio of transport scattering coefficients of the medium $\mu_s'(\lambda_1)$ and $\mu_s'(\lambda_2)$ at the two wavelengths.
2. A method as claimed in claim 1 comprising effecting said changes in the absorption coefficients by means of a common cause.
3. A method as claimed in claim 1 or claim 2 comprising effecting said changes in the absorption coefficient $\Delta\mu_a(\lambda_1)$ and $\Delta\mu_a(\lambda_2)$ by varying the concentration of an existing chromophore in the scattering medium.
4. A method as claimed in claim 1 or claim 2 comprising effecting said changes in the absorption coefficients $\Delta\mu_a(\lambda_1)$ and $\Delta\mu_a(\lambda_2)$ by adding a chromophore to the scattering medium.
5. A method as claimed in claim 3 or claim 4 wherein the ratio of the effected absorption coefficient changes $\Delta\mu_a(\lambda_1)$ and $\Delta\mu_a(\lambda_2)$ is determined from the ratio of the absolute chromophore absorption coefficients $\mu_{ad}(\lambda_1)$, $\mu_{ad}(\lambda_2)$.

6. A method as claimed in any preceding claim wherein the characteristic X measured in step (b) is the attenuation A of the light during its passage through the medium.
7. A method as claimed in any of claims 1 to 5 wherein the light is intensity modulated light, the measured characteristic X being the phase, ac intensity or depth of the modulation of the light through the medium.
8. A method as claimed in claims 1 to 5 wherein the light is a short pulse of light, the measure characteristic X being the time of flight (as mean time of transmission) of the light through the medium.
9. A method of determining the variation with wavelength of the ratio of absorption coefficients of a scattering medium (the relative spectral characteristic) comprising performing the method of any of claims 1 to 8 at a number of wavelengths across a waveband of interest, and in step (e) thereof determining the ratio of the absorption coefficients relative to that at a reference wavelength λ_r , the variation of said ratios with wavelength being the said spectral characteristic.
10. A method of determining the ratio of concentrations of chromophores of known spectral characteristic (as herein defined) in a light scattering medium comprising performing the method of steps (a) to (d) of any preceding claim at not less than n different wavelengths where n is the number of chromophores, one of said n wavelengths being the reference wavelength λ_r , performing step (e) to determine the ratio $\mu_s(\lambda_1)/\mu_s(\lambda_r)$ of the medium for each of the other wavelengths, and determining the ratio of concentrations of the chromophores in the medium from said ratios.
11. A method of determining the absolute concentration of chromophores of known spectral characteristic, as claimed in claim 9, where one of the chromophores is of known concentration.
12. Apparatus for determining the ratio of the absolute absorption coefficient $\mu_s(\lambda_2)$ and $\mu_s(\lambda_1)$ of a light scattering medium at two different wavelengths comprising

- a) means for passing light (as herein defined) through the medium;
 - b) means for measuring a characteristic X of the light at least two wavelengths λ_1 and λ_2 which is affected by the absorption coefficient during its passage through the medium;
 - c) means for effecting changes $\Delta\mu_a(\lambda_1)$ and $\Delta\mu_a(\lambda_2)$ in the absorption coefficient at the two wavelengths;
 - d) means for determining the ratio of the absolute absorption coefficients $\mu_a(\lambda_1)$ and $\mu_a(\lambda_2)$ at said two wavelengths from the said changes therein $\Delta\mu_a(\lambda_1)$ and $\Delta\mu_a(\lambda_2)$ or from quantities representative thereof, from the characteristic X , and from the ratio of transport scattering coefficients of the medium $\mu_s'(\lambda_1)$ and $\mu_s'(\lambda_2)$ at the two wavelengths.
13. Apparatus as claimed in claim 12 wherein the means (b) is adapted to measure the attenuation A of the light during its passage through the medium as said characteristic X .
14. Apparatus as claimed in claim 12 wherein the light is intensity modulated light and means (b) is adapted to measure the phase or the intensity or the depth of modulation of the modulated light through the medium as said characteristic X .
15. Apparatus as claimed in claim 12 wherein the light is a short pulse of light, and means (b) is adapted to measure the time of flight (mean time of transmission) of the light through the medium as said characteristic X .
16. Apparatus for determining the ratio of concentrations of chromophores of known spectral characteristic (as herein defined) in a light scattering medium comprising means performing the method of steps (a) to (d) of any of claims 1 to 11 at not less than n different wavelengths where n is the number of chromophores, one of said n wavelengths being the reference wavelength λ_r , means for performing step (e) to determine the ratio $\Delta\mu_a(\lambda_1)$ and $\Delta\mu_a(\lambda_2)$ of the medium for each of the other wavelengths, and means for determining the ratio of concentrations of the chromophores in the medium from said ratios.
17. Apparatus as claimed in any of claims 12 to 16 wherein the means (c) is arranged to effect said changes by a common cause.

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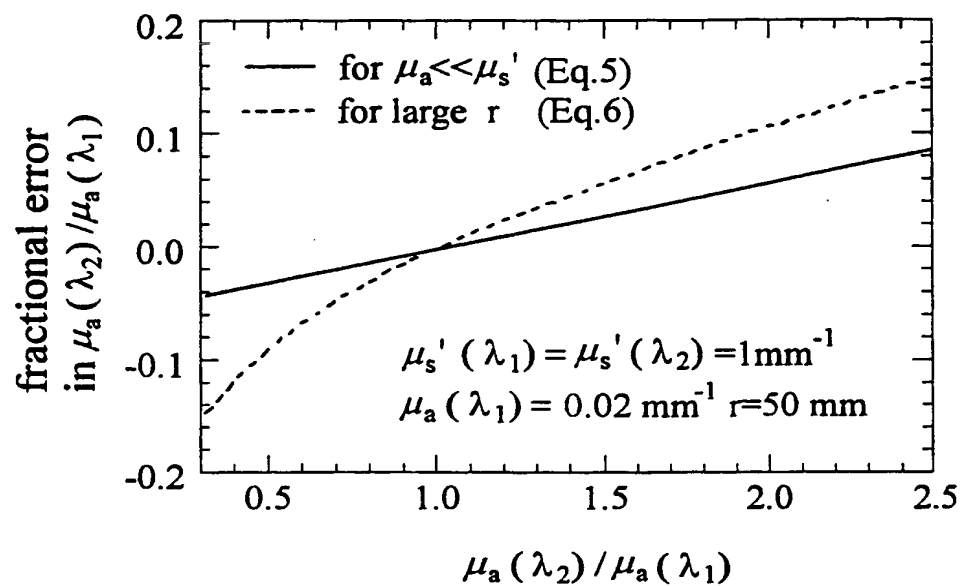


Fig. 1

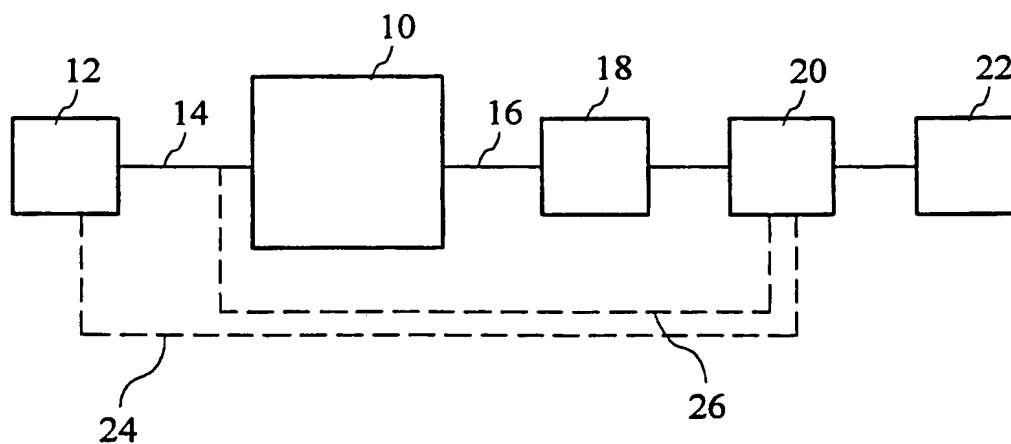


Fig. 5

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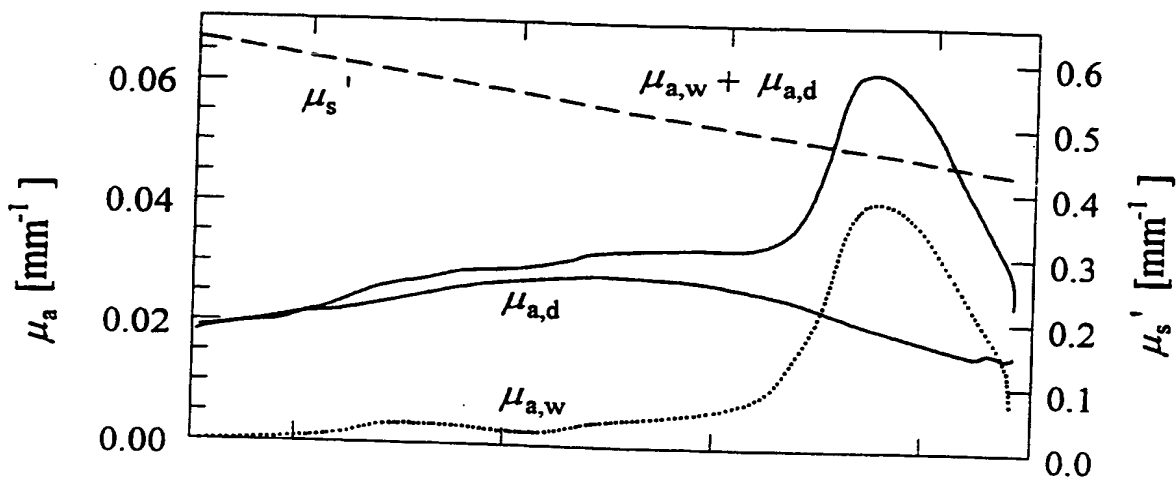


Fig. 2A

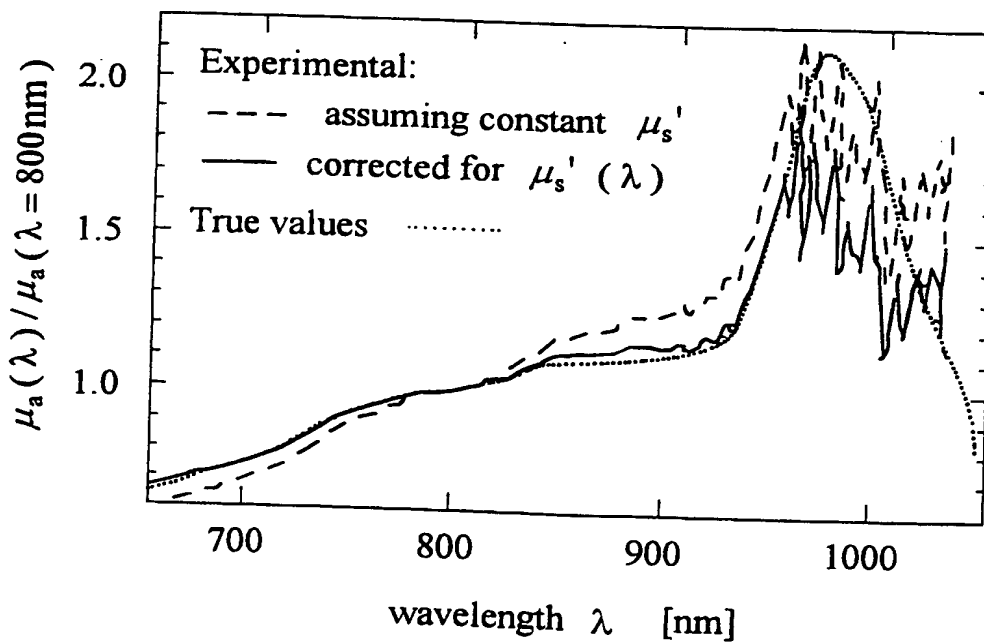
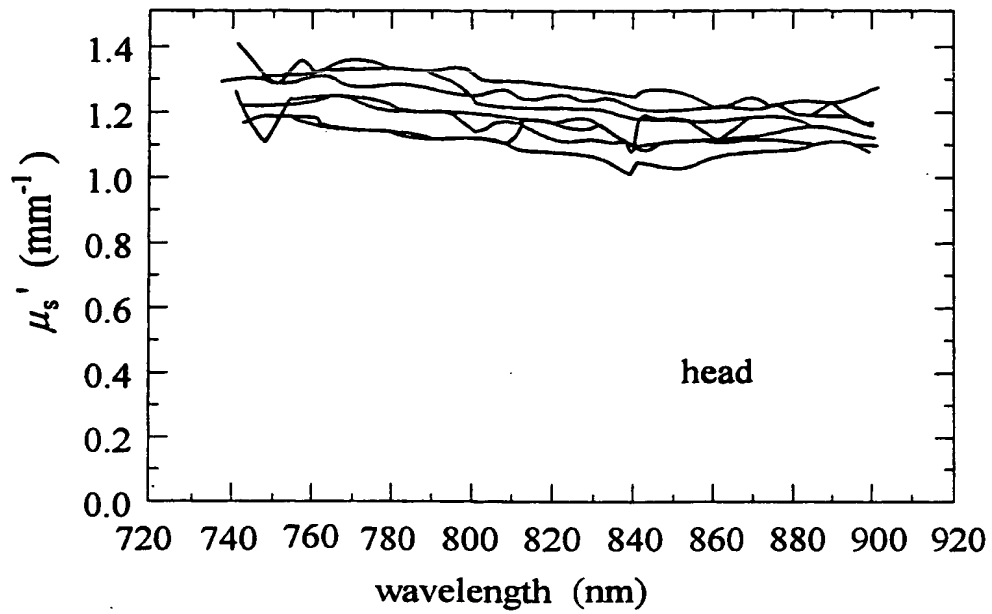
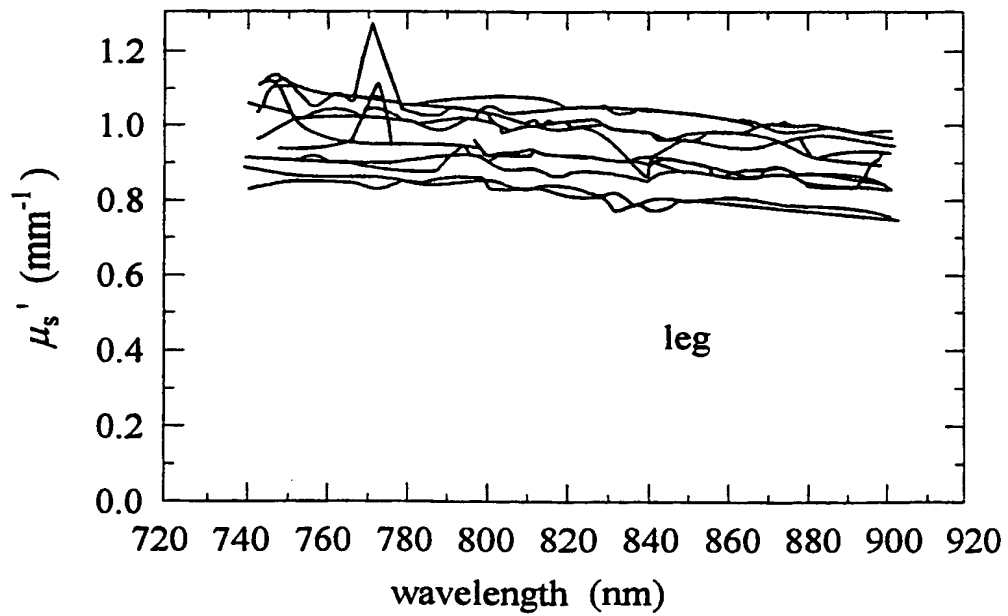


Fig. 2B

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*Fig. 3**Fig. 4*

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/00297

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N21/31

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	US 5 281 396 A (LEISSING) 25 January 1994 see column 7, line 46 - column 8, line 10 see column 8, line 36 - line 46 ---	1,3,6, 12,13
A	US 4 448 889 A (NERI) 15 May 1984 see column 1, line 25 - line 33 see column 1, line 43 - line 53 see column 3, line 23 - line 59 ---	1,6,12, 13
A	GB 2 030 696 A (SOUVANIEMI) 10 April 1980 see abstract see page 1, line 64 - line 85 see page 2, line 53 - line 59 see page 2, line 72 - line 75 ---	1,6,12, 13
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/00297

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
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